

### **In the Specification:**

Please amend the specification as follows:

On page 21, please amend the paragraph starting on line 14 as follows:

#### **-- 1.3 PCR**

Bisulfite converted genomic DNA was amplified with primers. It is preferred that said primers encompass nucleic acid sequences not comprising nucleotides which are formerly methylated. It is also preferred that said primers span regions comprising originally methylated or non-methylated nucleotides. In the present invention, for example, bisulfite converted genomic DNA was amplified with primers fully complementary to the deaminated DNA strands (forward 5'-TGAGTTGGAATAAGTTAGGGTAGATGTG-3' (SEQ ID NO:1); reverse 5'-CAACTCTCTATATCCCTTTCTAACATAAATCA-3' (SEQ ID NO:2)), yielding a product of 102 bp length. For the PyroMeth assay, the forward primer was biotinylated. The primers do not contain CpG dinucleotides so that the amplification step does not discriminate between templates according to their original methylation status. The following protocol was used for PCR reaction (modifications for the SNaPmeth application are indicated in brackets): the PCR reactions had a total volume of 50 µl (25 µl). 10 µl (5 µl) of agarose-embedded DNA were used as template DNA. The template DNA, 10 µM of each primer, 10 mM dNTPs, 0.4 U Ampli-Taq Gold Polymerase (0.2 U) were incubated with 5.0 µl reaction buffer (2.5 µl). The amplification was performed in a PTC 200 cycler from MJ Research under the program conditions 95 °C/10 min followed by 40 cycles of 95 °C/1 min, 58 °C/1 min, 72 °C/1 min, and an extension step at 72 °C for 5 min. For each sample, two independent PCR amplifications were performed and analysed. For PyroMeth, unincorporated primers and dNTPs were separated from the PCR product using the Invisorb PCR HTS 96 Kit (Invitek GmbH, Berlin, Germany). For the SNaPmeth assay, enzyme-based digestion of single stranded oligonucleotides and unincorporated

dNTPs was performed, using SAP and Exol according to the supplier's recommendation (Amersham, Braunschweig, Germany). --

On page 22, please amend the second full paragraph starting on line 19 as follows:

-- **1.5 MethylSNP analysis**

*SNaPmeth.* 1-3 µl of SAP- and Exol-treated PCR product (~ 0.15 pmol) were used for each SNaPmeth primer extension reaction. 0.5 pmol primer (5'-TTAGGGGGGTGAATATTGGG-3' (SEQ ID NO:3)) and 1.25 µl SNaPshot Ready Reaction Mix (including AmpliTaq DNA polymerase, fluorescence-labeled (F) ddNTPs, reaction buffer; PE Biosystems, Weiterstadt, Germany) were added to a total reaction volume of 10 µl. Cycling parameters: 96 °C/30 sec. 60°C/1 min, 25 cycles in a 96 well microtiter plate. Post-extension treatment with SAP (1 h, 37°C), removed the 5' phosphoryl groups of unincorporated [F]ddNTPs, prohibiting interference of fluorescence signals during electrophoresis. For electrophoresis on the ABI PRISM® 310 Genetic Analyzer POP-4™ polymer was used, with an injection time of 4 sec and a collection time of 13 min. The run files were analysed using GeneScan Analysis Software version 2.1. Peak area values were used to calculate allele frequencies in % (e.g. peak area C/peak area C+T) x100), representing the methylation grade at CpG no 7. The mean of the calculated allele frequencies of one sample was normalized to the calibration curve ( $y=mx+b$ , with y: observed allele frequency, m: regression coefficient as the slope of the function, x: expected allele frequency, b: intersection point of curve with zero). --

On page 23, please amend the first full paragraph starting on line 5 as follows:

-- *PyroMeth.* Single-stranded PCR fragments are needed for the sequencing-by-synthesis reaction. To purify the biotinylated PCR fragments ~~they~~ are immobilized on

streptavidin-coated Dynabeads® M-280 Streptavidin (DynaL A S, Oslo, Norway), according to the protocol of the SNP Reagent Kit 5x96, Pyrosequencing. After incubation for 15 min at 65 °C, the reactions were transferred into a PSQ™ 96 well reaction plate and denatured in 0.5 M NaOH, 10 min. The single stranded PCR fragments were captured with the magnetic rod, transferred in a PSQ 96 well plate and washed in 1x annealing buffer. Again transferred, the single stranded PCR fragments were hybridized with 10 pmol sequencing primer (5'-GGGGTGAATATTGGG-3' (SEQ ID NO:4)) in 1x annealing buffer, 80 °C for 2 min, then moved to room temperature. --